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by

Albert D. Venosa
Wastewater Research Division
Water Engineering Research Laboratory
Cincinnati, Ohio 45268

WATER ENGINEERING RESEARCH LABORATORY
OFFICE OF RESEARCH AND DEVELOPMENT
U.S. ENVIRONMENTAL PROTECTION AGENCY
CINCINNATI, OH 45268

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16. ABSTRACT

This paper is a brief literature review of the occurrence, distribution, and densities of bacterial indicators in raw and treated sludges. It discusses detection methodology and points out the serious needs for improvement in such methodology. It also briefly addresses the public health significance of numbers reported in the literature vis-a-vis the methods used to detect the organisms.

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DETECTION AND SIGNIFICANCE OF PATHOGENS IN SLUDGES

by

ALBERT D. VENOSA

INTRODUCTION

A large portion of the municipal wastewater sludge generated in the United States is currently being disposed on or in the land. This practice is expected to increase as more publicly owned treatment works (POTW's) are constructed or upgraded and as ocean dumping is finally phased out. Thus, a more complete knowledge of the potential risks to public health associated with such disposal is needed, especially vis-a-vis pathogenic microorganisms. Although a significant data base has been developed over the past 7 or 8 years on the survival and fate of bacterial contaminants in sludges and on sludge amended soils, our knowledge of potential health effects is still far from complete. The U.S. Environmental Protection Agency (EPA) is being hard pressed to issue specific guidelines regulating the use of wastewater sludges on the land, and the lack of a thorough understanding of the threat to humans posed by microbial pathogens has hampered the Agency from carrying out its responsibilities to municipalities.

Interim regulations relating to sludge treatment and disposal were published in the Federal Register in September, 1979. They appear in the current "Code of Federal Regulations (CFR, Title 40, "Criteria for Classification of Solid Waste Disposal Facilities and Practices," para. 257.3-6, Disease, Interim Final Regulations, effective October 15, 1979). Three degrees of restriction of sludge disposal are specified, depending on the degree of pathogen reduction. These are:

1. Raw sludge cannot be applied to, incorporated into, or injected shallowly into the land surface. The basis for this requirement is the potential hazard from the pathogens and also the need to minimize attraction of disease vectors by sludge deposited on or leaking to the land surface.

2. Sludges that have undergone treatment resulting in "significant reduction of pathogens" can be applied to or injected shallowly into the land surface subject to several restrictions: a. public access is controlled for at least 12 months; b. grazing by animals, the products of which are consumed by humans, is prevented for one month; and c. crops grown for human consumption that contact the sludges are not grown within 18 months after sludge application. The 18-month requirement is waived if the edible portion of crops does not contact the sludge.

3. Sludge that has undergone treatment resulting in "further reduction of pathogens" is not restricted for land use.

The definitions of significant reduction and further reduction of pathogens are vitally important to the application of the criteria. For a sludge treatment process to qualify as a "process to significantly reduce pathogens" (PSRP), it must produce a pathogen reduction at least equivalent to that obtained by good anaerobic digestion. The logic of the definition rests on the observation that agricultural use of anaerobically digested sludge as a fertilizer has been practiced for many years with no evidence that the practice has caused human illness, provided the digestion is adequate. Since these farming operations were on land with limited access and clearly defined use, this same restriction was applied to the use of PSRP sludge. Unfortunately, this definition is not based on sound scientific information related to the survival and transport of pathogens in sludge amended soils. Further, the paucity of documented health problems associated with the land application of sludges may reflect the lack of sufficiently sensitive epidemiological tools to detect small-scale incidents of disease.[1]

"Processes to further reduce pathogens" (PFRP) include composting, pasteurization, and treatment by electron beams or by gamma ray irradiation. A new process must perform in a similar way to these processes. Their common feature is that, when properly operated, they expose sludge to conditions that would essentially eliminate pathogens of concern.

The following processes qualify under the interim regulations as PSRP:[2]

- Aerobic digestion: The process is conducted by agitating sludge with air or oxygen to maintain aerobic conditions at residence times ranging from 60 days at 15°C to 40 days at 20°C, with a volatile solids reduction of at least 38%.

- Air drying: Liquid sludge is allowed to drain and/or dry on underdrained sand beds, or paved or unpaved basins in which the sludge is at a depth of 9 inches (22.9 cm). A minimum of 3 months is needed, 2 months of which temperatures average on a daily basis above 0°C.

- Anaerobic digestion: The process is conducted in the absence of air at residence times ranging from 60 days at 20°C to 15 days at 35°C to 55°C, with a volatile solids reduction of at least 38%.

- Composting: Using the within-vessel, static aerated pile or windrow composting methods, the solid waste is maintained at minimum operating conditions at 40°C for 5 days. For 4 hours during this period the temperature exceeds 55°C.

- Lime stabilization: Sufficient lime is added to produce a pH of 12 after 2 hours of contact.

- Techniques demonstrated to be the equivalent of the above on the basis of pathogen and volatile solid reduction.

TYPES AND NUMBERS OF BACTERIA IN RAW AND TREATED SLUDGES

This paper addresses the detection and significance of bacteria in sludges and soils. Other workshop participants are addressing viruses and parasites.

There appears to be a general consensus among contributors to the literature that the bacterial pathogens of primary concern in sludges are as listed in Table I.[1,3,4]

TABLE I. Bacterial pathogens in sludge and their associated diseases

Organism	Disease
Campylobacter jejuni	Gastroenteritis
Escherichia coli (enteropathogenic strains)	Gastroenteritis
Leptospira sp.	Leptospirosis, infectious jaundice
Salmonella spp.	Gastroenteritis, enteric fever
Shigella spp.	Acute dysentery
Vibrio cholerae	Cholera
Yersinia spp.	Yersiniosis, gastroenteritis

All species listed may produce symptomless infections and many have important non-human reservoirs as well.

Enteric pathogens are discharged to municipal wastewater in the feces of infected individuals. These pathogens become associated with sludge during wastewater treatment because they remain in their original solids-bound state, they co-sediment along with other particulates, and they have an affinity for the particulate fraction of wastewater that ends up as sludge. The concentration of a given enteric pathogen in sludge varies with the disease morbidity in the area served by the treatment plant. Some pathogens are found in sludge at fairly constant levels through the year, while the concentrations of other pathogens vary on a seasonal basis. Variability in the levels of fecal indicator organisms (total coliforms, fecal coliforms, fecal streptococci) is significantly lower than pathogen variability because the former are the common intestinal inhabitants of the general population. Pedersen[2] presented data on the density levels of indicators and pathogens in raw primary, secondary, and mixed sludges, and these data are summarized in Table II.

Anaerobic Digestion

When sludge is anaerobically digested at mesophilic temperatures, a 1- to 3-log reduction in indicators and pathogens has generally been observed.[2,4] The literature values have been highly variable, however. A major factor affecting the variability of

TABLE II. Density levels of bacteria in raw sludges (average geometric mean of organisms per gram dry weight)[2]

Organism	Primary	Secondary	Mixed
Total coliforms	1×10^8	7×10^8	1×10^9
Fecal coliforms	2×10^7	8×10^6	2×10^5
Fecal streptococci	9×10^5	2×10^6	4×10^6
Salmonella spp.	4×10^2	9×10^2	3×10^2
Pseudomonas aeruginosa	3×10^3	1×10^4	3×10^3

anaerobic digestion in removing bacteria is the method by which the digestor contents are drawn and replenished. Some digestors are routinely filled before removal of the day's sample. Even if the mixing is adequate, some of the microbial product being removed is fresh sludge that has just been added. The detrimental effect of this practice is dramatically illustrated in Figure 1, which compares the total coliform levels in two identical digestors, each receiving the same feed sludge but differing in the way in which the sludge is added and removed (unpublished data from J. Rickabaugh and D. Freedman, University of Cincinnati). The mean total coliform log reduction in the digestor operating in the fill and draw mode is 1.38, while that in the draw and fill mode is 2.77, a difference of 1.39 log units. Clearly, the fill and draw mode is a poor practice.

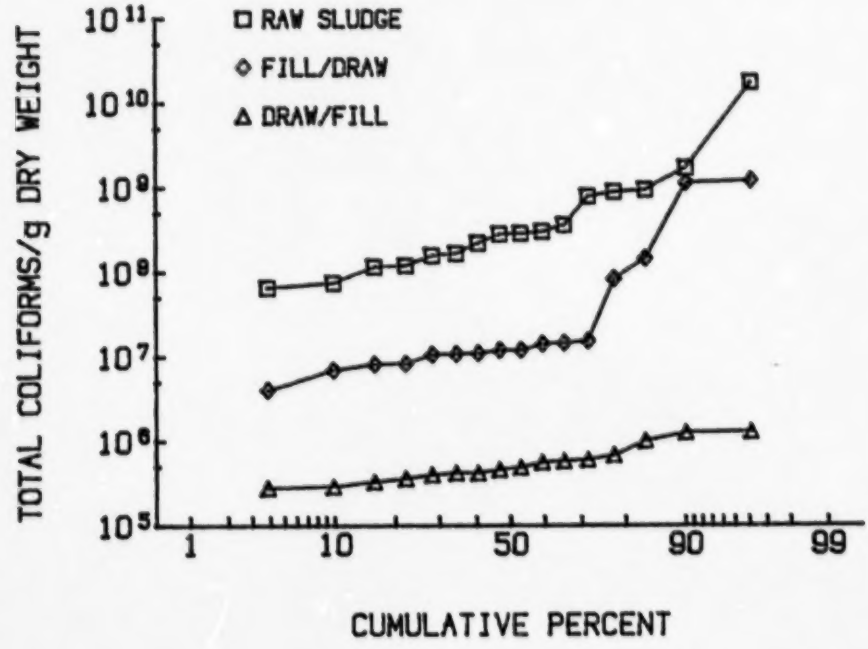

Figure 1. Frequency distribution of total coliforms in anaerobic digestors operating in fill and draw vs. draw and fill modes.

Two other factors of importance affecting bacterial die-off in anaerobic digestors are detention time and temperature. Generally, the longer the detention time, the greater the die-off.[5] Temperature, however, is the primary controller in the die-off of indicators and pathogens. At 49°C, 1- to 3-log increases over reduction at 35°C were reported by Berg and Berman[6] and Stern and Farrell.[7]

Aerobic Digestion

According to Farrah and Bitton,[8] when sludge is aerobically digested at mesophilic temperatures, a maximum 1- to 2-log reduction in indicators and Salmonella spp. can be expected. Bacterial removals in three local digestors operating at mesophilic temperatures and detention times ranging from 17 to 50 days were of that magnitude. One of the reasons why only 1- to 2-log reductions were observed was that the digestors were completely mixed and continuously fed, so that sludge leaving the digestor always contained a small amount of freshly added, undigested sludge. Another reason is that most aerobic digestors operate as open vessels, so that any heat produced from the exothermic oxidative reactions in the digestor is lost to the atmosphere. If the digestor were a closed tank, so that the heat can be trapped and allowed to build up, much greater log-reductions would be expected to occur. Indeed, Kabrick and Jewell[9] did observe that a full-scale autoheated aerobic digestor that routinely achieved temperatures in excess of 45°C inactivated pathogens to a significantly greater extent than anaerobic digestors operating at longer detention times. In fact, if temperatures can be maintained at 55°C for 10 days and volatile solids reduced by 38%, aerobic digestion would qualify as a PFRP.

Composting

To qualify as a PSRP, composting for at least 5 days can be conducted by the aerated static pile, windrow, or within-vessel method provided that the minimum temperature of 40°C within the compost mass is maintained throughout the composting period and temperatures > 55°C are attained for at least 4 hours of the composting period.[2] Composting at temperatures of 55°C or greater for 3 days (within-vessel or aerated pile methods) or 15 days coupled with a minimum of 5 turnings (windrow method) qualifies as a PFRP.

Temperature is the key factor affecting survival of bacteria in composting operations. Higher temperatures are more consistently and reliably achieved in aerated static piles than in windrows.[2] That is why Burge et al.[10] observed only a 3-log decrease in total coliforms after 15 days in a windrow, although no Salmonella survived. Although composting is effective in reducing pathogenic bacteria to very low levels, regrowth can occur, even in material that consists of as much as 60% solids. Brandon et al[11] inoculated sterile composted sludge with Salmonella enteritidis. After 2 days incubation, the bacterial counts were greater than 10^9/g. In other studies by Brandon's group,[12] competitor organisms were shown to have significant effects on bacterial regrowth potential,

and moisture content > 20% was necessary to support regrowth. The latter finding was confirmed by Yeager and Ward[13] and Russ and Yanko.[14]

Clearly, then, composting operations approaching thermophilic temperatures can achieve fairly rapid and complete bacterial inactivation. No viable Salmonellae, fecal coliforms, or total coliforms were found after 10 days of aerated static pile composting[10] or mechanical composting.[15]

Air Drying

Air drying is usually performed on sludge that has undergone some form of digestion previously. Because temperatures can be quite low during air drying, the most significant factors affecting pathogen survival are the state of dryness (moisture content) and detention time at a given moisture level.[4] Ward et al.[16] found air drying had only a marginal effect on bacterial survival unless the sludge was nearly fully dry. In a study by Yeager and Ward,[13] bacterial survival in partially dried sludges, held at 21°C in sealed containers to prevent evaporation, decreased in proportion to the amount of dryness at which the sludge samples were maintained. In sludge with greater than 90% solids, bacteria survived as well as in liquid sludges.

Lime Stabilization

Lime stabilization of sludge qualifies as a PSRP if sufficient lime is added to produce a pH of 12 after 2 hours of contact. The key factor affecting bacterial survival is pH. If the pH of the sludge drops significantly below 12 (and this can easily occur in short period of time) regrowth will occur, thereby negating the loss of viability previously achieved.

Lime stabilization can be very effective in reducing bacterial numbers in sludge.[17] Typical decreases in indicator levels are in the range of 3 to 6 logs within 2 hours at pH 12. The treatment, however, does not prevent regrowth, because it does not affect the availability of food to the microorganisms.

A summary of the levels of bacterial indicators in the PSRP sludges mentioned above is presented in Figure 2. Results presented do not take into account regrowth.

SURVIVAL IN THE ENVIRONMENT

In 1975, Gerba et al.[18] discussed the factors affecting the survival of enteric bacteria in surface soil. These are:

1. Moisture content. Moist soils and periods of high rainfall increase survival time.

2. Moisture-holding capacity. Survival time is shorter in sandy soils than those with greater water holding capacity.

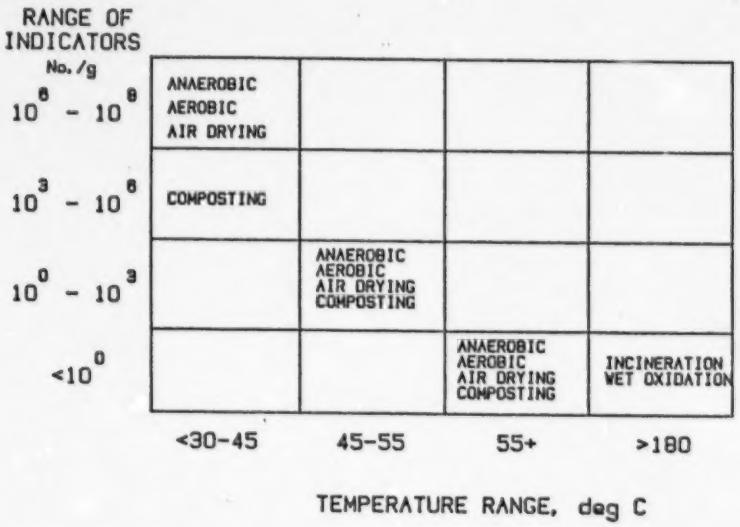

Figure 2. Expected indicator levels in various digestion processes as a function of digestor temperature.

3. Temperature. Survival time is longer at lower temperatures.

4. pH. Survival times are shorter in acid soils (pH 3-5) than in neutral or alkaline soils. The high level of fungi in acid soils may play a role.

5. Sunlight. Survival time is shorter at the surface, probably due to desiccation and high temperatures as well as ultraviolet radiation.

6. Organic matter. Presence of organic matter increases survival time, in part due to its moisture-holding capacity. Regrowth of some bacteria may occur. In highly organic soils, anaerobic conditions may increase the survival of Escherichia coli.[19]

7. Soil microorganisms. Competition, antagonism, and predation decrease survival times. Enteric bacteria applied to sterilized soil survive longer than those applied to unsterilized soil.

In a more recent review Gerba[1] suggested that both survival and soil retention of bacteria are largely determined by climate (temperature and rainfall), nature of the soil, and nature of the microorganism. Bacterial die-off rates were found to approximately double with each 10° rise in temperature between 5 and 30°C (Reddy et al.).[20] Rainfall mobilizes previously retained bacteria and greatly promotes their transport in groundwater. Several workers have shown that the greatest degree of drinking water contamination occurs after periods of heavy rainfall.[21-23]

Soil properties influence moisture-holding capacity, pH, and organic matter, all of which can control bacterial survival in soil. Soil properties such as particle size, cation exchange capacity, and clay content will influence retention.[1] Removal of bacteria by soil is believed to be largely due to mechanical removal (filtration at the soil surface) and adsorption to soil particles. Bacteria are largely removed within about 50 cm of soil surface. Transport through the soil matrix tends to be greater in coarse sand or gravel soils. Adsorption to soil particles increases with the presence of clay-sized particles, high cation concentration, and low pH. This adsorption is reversible, and bacteria can be released and moved down the soil profile by rainfall events that lower the conductivity of the soil water (Lewis et al.).[22]

Kowal[24] recently summarized information on bacterial penetration of soil treated by overland flow and slow rate systems (wastewater, not sludge, application). He suggested that bacteria may penetrate 10 m vertically and up to 450 m laterally. These distances are highly site-specific, and travel distances are generally less than the maximum reported.

A summary of survival times of bacteria in soil (Table III) was prepared by Kowal,[24] who was summarizing data from Bryan[25] and Feachem et al.[26]

TABLE III. Survival times of bacteria in soil[24]

Organism	Survival time, days
Fecal coliforms	8 - 55
Fecal streptococci	8 - >70
Leptospira	<15
Mycobacterium spp.	10 - 450
Salmonella spp.	11 - >280

The term "survival" is used in a qualitative sense to mean ability to be detected. Since inactivation is a rate process, detection depends on the initial level of organisms, sensitivity of detection methodology, and other factors. Clearly, the values reported in the literature vary widely, and one of the reasons for the observed variation is detection methodology, which is the subject of the next section of this review.

DETECTION METHODOLOGY

Bacterial numbers reported in the literature are only as good as the methodology used to detect them. Conventional most probable number (MPN) and membrane filtration (MF) methods are used to detect indicator organisms (total and fecal coliforms, fecal streptococci) in sludge, and there is a growing need to develop methods for the direct isolation and enumeration of bacterial pathogens of emerging significance, such as Yersinia enterocolitica and Campylobacter jejuni. With respect to detection of salmonellae, there is no "standard" method used by all re-

searchers. In fact, it is evident from the literature that there are almost as many methods as there are investigators.

The problem faced by any analyst attempting to quantify bacterial numbers in sludge is the ability to achieve homogeneity in the sample and to separate the organisms from the solid particles to which they are adsorbed. There has not been any extensive comparison of solids elution procedures published to make any one method preferable over another. In 1980, Dudley et al.[27] compared sonication, blending, and vortex mixing with glass beads and found the latter method gave the highest numbers of indicators and heterotrophs in secondary and digested sludges. No data on precision were presented, however, so it is premature to conclude that vortex mixing is the preferred method. On theoretical grounds, one could argue that such a mixing regime is certainly milder than blender homogenization, and if further research were to find the inherent precision acceptable, then consideration should be given to adopting such a sample handling procedure as standard.

Following sample handling, the next decision the analyst is faced with is whether MPN, MF, or direct plating on solid medium should be used. Usually, with raw sludge, where the numbers of indicators are expected to be high, serial dilutions of the sample will result in elimination of solids as an interference problem. Thus, it does not matter which of the three inoculation procedures is used. If, however, the sludge being analyzed is a digested sludge, in which the numbers of bacteria to be quantified are expected to be substantially lower, solids will interfere with the MF procedure. Then, one is faced with deciding whether to use the MPN or direct plating. Here, the field appears to be mixed, with a slight edge being given to direct plating onto the appropriate selective agar medium.[8,27]

The final decision that needs to be made is the proper selective medium and incubation temperatures to be used. Here, there is a wide variety that exists in the literature. Pedersen[2] summarized no less than 20 methods in use for indicators and 30 for pathogens. Clearly, a standardized test is needed to bring order out of chaos, and until such standardization is achieved, variability in reported numbers will never be reduced.

PUBLIC HEALTH SIGNIFICANCE

No review of pathogens in sludge would be complete without a brief discussion of infective dose and risk of contracting disease upon exposure to the sludge. A brief summary of infective dose data of representative bacterial pathogens is presented in Table IV.[25]

Clearly, dose-responses are quite variable in humans, and the onset of infection is dependent on a whole host of factors. For example, the effect of stomach acidity on the dose of cholera organisms needed to cause infection is evident, the dose being decreased by over 3 orders of magnitude by buffering. Other factors affecting dose-response are level of immunity, age, and general health of the exposed population.

TABLE IV. Infective dose of enteric bacteria[25]

Bacterium	No Infection	Percent of Volunteers			
		1-25	26-50	51-75	76-000
Escherichia coli.	10^4	10^6	10^8	10^8-10^{10}	10^{10}
Salmonella typhi.	10^3	10^5	10^5-10^8		10^8-10^9
Salmonella spp.	10^4-10^6	10^6	10^7	10^7-10^8	
Shigella		10^1-10^2	10^2-10^4	10^3	10^4
Vibrio cholerae					
Buffered	10^1		10^3-10^8	10^4-10^6	
Unbuffered	10^4-10^{10}		10^8-10^{11}		

The risk of infection is probably greatest for Salmonella and Shigella spp., because they are the most common bacterial pathogens in municipal wastewater.[24] It is generally accepted that the minimal infective dose of Salmonella is in the range of 10^5-10^8, which was originally reported in the early 1950's in prisoner volunteer studies.[28-30] However, some recent data indicate that infective levels may be substantially lower than that. Imitation ice cream contaminated with three Salmonella serotypes caused an estimated 9000 cases of gastroenteritis in New York City in 1967.[31] Analysis of leftover samples of the incriminated ice cream indicated that individual servings may have contained 10^4 salmonellae. A similar inoculum, 15,000 S. cubana, was estimated to produce gastroenteritis in persons exposed to carmine dye while being evaluated for gastrointestinal disorders.[32] In 1973 and 1974, chocolate contaminated with S. eastbourne was epidemiologically incriminated in more than 200 cases of illness reported in Canada and the U.S.[33] Analysis of the chocolate suggested that an inoculum of no more than 1000 organisms (the number in a one-pound bag) was sufficient to cause illness. Several factors may have accounted for this finding, including technical difficulties in quantitative recovery of salmonellae from chocolate, the youth of the patients in this outbreak, possible protection of the Salmonella organism by the fat in chocolate against the action of gastric acid, and possible greater virulence of the epidemic strain of S. eastbourne compared with that of other serotypes previously studied. One other point should be made with respect to this outbreak that may have a bearing on the risk assessment of salmonellae in sludge. Laboratory investigations[34] supported the concept that the dryness of chocolate (less than 17% moisture) conferred enough heat resistance to salmonella strains to survive exposure to temperatures of 60°C for 10 hours.

Although the high infective dose for Salmonella is unlikely to persist in sludge-amended soils for extended periods of time, the dose might be reached on a contaminated foodstuff under conditions that allow multiplication.[24] This, coupled with the low infective dose for Shigella, prompted Kowal to conclude that

".....it would be prudent for humans to maintain a minimum amount of contact with an active land treatment site, and to rely on 'time' to reduce the bacterial survival...when growing crops for human consumption."

SUMMARY AND DISCUSSION

This paper summarized current knowledge on the occurrence, distribution, and densities of bacterial indicators and pathogens in raw and treated sludges and discussed detection methodology and public health significance of bacterial pathogens in sludges. It was by no means meant to be an exhaustive literature review on the subject, but merely a short overview of the problem.

To summarize, the kinds and concentrations of indicator and pathogenic bacteria in sludges have been documented many times in the literature. Densities range from 10^6 to 10^8 per gram dry weight for indicator bacteria and 10^1 to 10^4 for pathogens. Bacterial reductions of 0.5 to >4 orders of magnitude have been reported for anaerobic digestion, aerobic digestion, air drying, composting, and lime stabilization processes. Scattered data are available on transport and fate of bacteria at disposal sites such as landfills and land application sites. At all sites, pathogen densities may be reduced by dilution, temperature, moisture, pH, and antagonistic or competitor organisms.

Detection methodology both for indicators and pathogens needs to be improved and standardized. Variability in literature values is high, and one factor that undoubtedly contributes to this variability is the methodology used to quantify the proper levels. Standardization in sampling preparation and handling, media selection, and mode of enumeration (MPN, MF, or direct plating) will help reduce this variability and enable better interpretations of the meaning of the results.

Finally, with respect to public health significance of pathogenic bacteria in sludge, Kowal[24] aptly concluded: "Although the infective doses of bacteria appear to be fairly high,it would be wise for humans to maintain a minimum amount of contact with an active land treatment site."

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Albert D. Venosa is a research microbiologist with the U.S. Environmental Protection Agency in Cincinnati, Ohio. Correspondence should be directed to Albert D. Venosa, U.S. Environmental Protection Agency, 26 West St. Clair Street, Cincinnati, OH 45268.

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